1015-Plat
Probing the Conformation Landscape of the Unfolded State: Do Disordered and Unfolded Dynamics Differ?
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Intrinsically disordered proteins undergo conformational changes that are often beyond the scope of current computational techniques used to study the dynamics of folded proteins. New approaches to define a metric for the dynamics of disordered proteins have been developed which are also readily applicable to the study of non-equilibrium globular protein dynamics. We use dimensionality reduction and clustering techniques applied to molecular dynamics (MD) simulations of a class of entirely disordered proteins (outside of a small anchoring domain) involved in nucleocytoplasmic transport, the FG-nucleoporins (FG-nups), as well as folding simulations of several globular proteins of similar size and sequence composition to compare disordered protein dynamics to early-stage folding dynamics. Our results provide detailed maps of the protein conformation space, allow us to classify proteins based on their dynamics, and indicate that disordered protein motion is of higher-dimensionality than early-stage folding dynamics.

1016-Plat
Generating Context-Specific Functions with Intrinsically Disordered Domains
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During animal development, Hox transcription factors specify many different tissues, including organs, appendages, and portions of structures that span the length of the animal, such as the nervous system, musculature, and ectoderm. Consequently, Hox proteins must sense the cellular identity and respond by instigating the appropriate gene regulatory program. To further complicate matters, Hox proteins interact with DNA via a homeodomain, which binds with high affinity yet notoriously low specificity. Using the *Drosophila* Hox protein Ultrabithorax (Ubx) as a model system, we have discovered that most of the Ubx sequences outside of the homeodomain are intrinsically disordered and regulate DNA binding. An intrinsically disordered region near the homeodomain modulates both DNA affinity and specificity. This region also mediates interactions with the Hox cofactor Extradenticle, and is alternatively spliced. Consequently, Extradenticle availability and alternative splicing may control DNA site selection. Ubx also must regulate genes in a position-specific manner within a single tissue. A second large intrinsically disordered domain modulates Ubx affinity and is required to bind transcription factors controlled by the WNT and TGFb/BMP cell signaling cascades. Since these signaling pathways subdivide Ubx-specified structures, this mechanism may control position-specific gene regulation within a field. Finally, once a Hox protein has bound DNA, it must select whether to activate or repress transcription. We find DNA binding by Ubx induces a conformational change, which increases the solvent exposure of the activation domain. We are currently investigating whether this conformational change allows DNA sequence to dictate the mode of transcription regulation. Using our carefully developed methods to generate and assay soluble, full-length *Drosophila melanogaster* Hox protein Ubx, we are identifying the mechanisms that drive context-specific Hox function in response to increasingly spatially restricted cues (tissue identity, location within a tissue, and DNA binding sequence).

1017-Plat
The Solution and Binding Behavior of the Intrinsically Disordered FG Nups Determined by STINT-NMR
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The Nuclear Pore Complex (NPC) mediates all transport between the nucleus and cytoplasm. The channel of the NPC is lined with ''FG Nups'', a family of intrinsically disordered proteins characterized by phenylalanine-glycine repeat motifs. FG nups form the exquisitely selective filter of the NPC; non-binding proteins are excluded while the binding of transport factors to the FG Nups facilitates their passage through the NPC. Like other intrinsically disordered proteins, the FG Nups appear to be very sensitive to their environment, showing vastly different behavior in different experimental conditions; in vitro, the observed behavior of the FG Nups varies from rigid gels to flexible random-coil polymers. We used STINT-NMR to probe the behavior of a model FG Nup within a living cellular environment. In a STINT-NMR experiment, NMR observations are directly performed on bacteria in vivo co-expressing a labeled protein and an unlabeled binding partner. We have found that the solution state of the FG Nup within living cells is completely disordered, while NMR spectra are significantly changed in vitro buffers, presumably from numerous intra- or inter-molecular contacts. Moreover, the binding interface between transport factors and the FG Nup differs considerably between solution and cellular conditions. Thus, a key determinant to FG Nup behavior is the local environment.
These results indicate that the proper behavior of the FG Nups is dependent on the normal cellular milieu, and is not necessarily represented in vitro; our findings have important implications for the various current models regarding the molecular mechanisms of nucleocytoplasmic transport and behavior of weak cellular interactions generally.
This work was supported by a Charles Revson postdoctoral fellowship, NIH F32 GM087854, NIH RO1 GM62428 (MR) and P41 GM66354(DC).

PLATFORM Y: Membrane Fusion

1018-Plat
Visualizing Release of Single Fluorophores at Membrane Fusion Sites
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SNARE proteins play a central role in nearly all intracellular fusion reactions; fusion is driven by formation of trans-SNARE complexes (SNAREpins) through pairing of vesicle-associated v-SNAREs with complementary t-SNAREs on target membranes. We recently reported a docking and fusion assay in which single small unilamellar vesicles containing the synaptic/exocytic v-SNAREs VAMP/synaptobrevin (v-SUVs) fuse rapidly with planar, supported bilayers containing the synaptic/exocytic t-SNARE syntaxin-SNAP25 (t-SBLs), with single fusion events occurring in ~130 ms after docking. We optimized acquisition conditions such that now the release of single fluorescently labeled lipids can be visualized. As the fluorophores diffuse away from the release site, individual spots become discernible and can be tracked with ~16 ms resolution. More than 90% of the tracked spots bleach in a single step, strongly suggesting they represent single fluorescent lipid molecules. Intensity-drops as spots disappear follow a normal distribution whose mean defines the intensity of a single fluorophore. This allows us to estimate the total number of lipids in a vesicle prior to its fusion with the t-SBL, given the label density. An independent estimate of vesicle size is based on extrapolating the number of surviving spots as a function of time to just before fusion.
We studied the effects of lipid composition. In bilayers containing nearly physiological amounts of cholesterol, the mean squared displacement of single fluorescent lipids that are released into the t-SBL increases linearly in time indicating a diffusive process and yields a diffusion coefficient of ~0.3 $\mu m^2/s$, a value that is similar to those found for lipid diffusivities in the plasma membrane of live cells. In addition, the majority of the vesicles that dock end up fusing. In contrast, in bilayers devoid of cholesterol, lipid diffusion is much faster, but only 40-50% of docked vesicles fuse.

1019-Plat
Synaptotagmin Expands Membrane Fusion Pore by Facilitating SNARE-Complex Formation
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Pore expansion, an essential step for SNARE-mediated membrane fusion, has not been well studied due to the lack of a reliable content mixing assay. Recently, we have developed a new assay to detect the inter-vesicular mixing of large cargoes at the size of several nanometers at the single molecule and vesicle level [1]. Through our new assay, we found that the neuronal SNARE complexes alone, without regulatory proteins, are able to expand fusion pore inefficiently. By interacting with t-SNARE proteins through a *trans*-conformer [2], membrane anchor synaptotagmin 1 and Ca^{2+} facilitate SNARE complex formation for fusion pore expansion.
References
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1020-Plat
Extracellular Ca^{2+} Directly Inhibits Exocytosis in Neurons
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